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Understanding Single-Cell Technology in clinical research applications

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"Single-cell technologies are driving biological research and clinical applications to evolve -from bench to bedside-" explains Dr. Irene Whitney, Director of Applications and Collaborations, Honeycomb Biotechnologies, United States

Single-cell analysis technology has contributed significantly to medical research. In the past few years, single-cell RNA sequencing technologies have been widely used for analyzing cancer constituent cells, identifying cells causing therapeutic resistance, and analyzing gene signatures of resistant cells.

Since the publication of the first single-cell RNA-sequencing (scRNA-seq) study nearly 15 years ago, a "cellular resolution revolution" has propelled single-cell technologies from academic labs into more mainstream discovery, development and clinical research applications. Understandably, the swift uptake and rapid pace of technological innovation in this field since then can seem overwhelming. Would-be single-cell researchers have myriad options and opportunities to choose from when organising their studies. To begin to make sense of this burgeoning field of study, a better understanding of the origins of these technologies, common challenges associated with single-cell studies and specific capabilities to look for in tools for single-cell isolation and analysis is an ideal starting point.

The Origins of Single-Cell Technology

To begin to understand current applications and future opportunities for single-cell technology, it can be helpful to first understand the technologies that preceded scRNA seq. One of these is bulk RNA sequencing – a method for transcriptomic analysis that measures the average expression of pooled cell populations, tissue sections or biopsies. This approach offers researchers a comprehensive look at a given sample, but it lacks the ability to drill down to the single-cell level.

Flow cytometry is another early technology used for single-cell research and is the basis for fluorescence-activated cell sorting (FACS). This technique is used to isolate individual cells from a sample into cell populations, labeling each with fluorescent markers and sorting them based on the color of the marker. This method allows researchers to analyse the properties of each population individually. While this approach offers a more granular look at cell properties, it is not as comprehensive as others.

In contrast to the aforementioned methods, scRNA-seq is both comprehensive and high-resolution. It allows for the transcriptomic analysis of heterogeneous tissue or dynamic processes in one single experiment – offering researchers the expression profiles of individual cells.

Challenges in Single-Cell Studies

Building on this foundational knowledge of technologies used for single-cell research, awareness of the potential pitfalls in these studies is also helpful to know. Inherently, there are technical complexities involved in working at the single-cell level. Across all applications, two factors are of the utmost importance: sample quality and sample preparation.

In terms of sample quality, fresh samples are considered the gold standard for single-cell research. Because RNA and protein expression changes rapidly in cells once they are removed from a host, efficient sample processing as soon as possible after collection is ideal. This is particularly challenging in multi-site studies where samples are being collected at multiple, sometimes remote locations, which will then require careful storage and shipping to maintain sample quality. Even still, fresh samples may not be available in all cases. In these instances, lower quality samples will suffice, but may end up hindering the final data outputs being analysed.

A second key challenge is sample preparation. Once a sample has been collected and processed, sample preparation can become an unintended source of technical variation and batch effects due to cell dissociation. Any enrichment required by rare cell types could also skew data and results yielded. Fortunately, these risks can be reduced or avoided altogether with sample prep best practices. For example, having an automated cell counter that is accurate and has two color fluorescence for live/dead markers (such as acridine orange/propidium iodide) is essential for rapid QC and optimisation of sample prep to create a protocol that provides the best possible viability.

Innovative Solutions for Single-Cell Research

In addition to following best practices for sample collection and preparation, scRNA-seq technology developers are innovating where research efficiencies are needed most. Sample preservation is one of these areas.

In many cases, researchers will store single-cell samples via cryopreservation – the process of collecting and storing cells, tissues, or organs at freezing temperatures for later use. While it’s a commonly used method, it may also cause undue stress and damage the sample. This is especially true for more fragile sample types such as clinical biopsies, or cell-types such as granulocytes, hepatocytes, and neurons. For instance, Honeycomb Biotechnologies is helping researchers circumvent these limitations with its flagship offering, the HIVE™ scRNAseq Solution, through the gentle isolation and capture of single cells combined with stable single-cell storage after capture. Single cells can be pipetted directly into handheld HIVE devices and the RNA of captured cells is preserved to lock in molecular signals. Cell-loaded HIVEs can then be stored until ready for Honeycomb’s scalable workflow for making single-cell RNAseq libraires. By preserving the sample at time of collection, the

HIVE solution maintains the full diversity of cells in a sample, who's transcriptomes can then be extracted, amplified and analysed at a later date.

Transportation is another key challenge for single-cell researchers and one that Honeycomb Biotechnologies has also aimed to solve. Different sample types, storage times and variable shipping protocols add complexity to how and when single-cell samples are moved from collection site to the lab for processing. Stable storage and shipping of cell-loaded HIVEs enables time-courses, sporadic or end-of-day samples, distributed collection sites with centralised processing, and multi-site collaborations. Without the need for specialised equipment, meaning the workflow can be run in most labs. This alone expands opportunities for researchers pursuing broader studies, especially those in lower resource settings.

Future Opportunities for Single-Cell Research

Despite some of the challenges involved, innovation in scRNA-seq technologies continue to make vast and varied applications of single-cell research possible. One field seeing increased interest and activity is innate immunology. These studies involve the capture of fragile cell types, such as neutrophils – the white blood cells playing a central role in inflammatory responses. Another booming area of single-cell research is in the field of infectious diseases, where the careful handling and storage of samples is of the utmost importance. Additionally, meaningful single-cell discoveries are being made in the arena of clinical research using human samples and minimally invasive biopsies.

Looking ahead, we can expect single-cell applications to continue moving "from bench to bedside," especially with further innovation in related technologies. The develops of these tools – whether they're in industry or academia – will continue to find ways to optimise research efficiencies and reduce the cost of performing these studies. Ultimately, the deeper understanding and democratisation of tools used to complete them will result in new discoveries that improve human health.

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